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(6) CHEMOTHERAPY OF MALARIA.

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19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

P. berghei blood-induced malaria infection in mice - based on mortality.

P. gallinaceum sporozoite-induced malaria infection - Aedes aegypti - chicks - based on mortality.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Primary screen - quantitative evaluation of potential antimalarial activity.

Primary screen - to provide quantitative assessments of prophylactic values.

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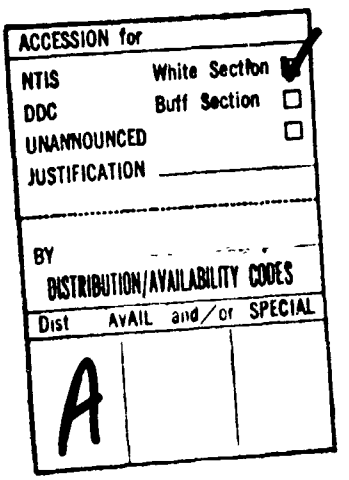
Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.



40,465 compounds were screened for antimalarial activity in the period from June 1, 1967 through May 31, 1968.

Our contractual commitment under Contract No. DA-49-193-MD-2218 was the screening of 37,500 compounds. However, in keeping with the policy set by WRAIR, the number specified in a contract is considered as the minimum. This year, as in past years, the final number of compounds screened has exceeded the minimum indicated.

Since our new quarters were not ready for occupancy until February 1, 1968, we continued to function in our old quarters during the first eight months of this contract year. Optimum points of efficiency and safety had long been passed, and the limitations and difficulties endured might have presented serious problems if our test system had been more complicated or less flexible.

Tables 1, 2, 3 and 4 list, month by month, the number of compounds tested and the number of mice used in the tests from June 1, 1964 through May 31, 1968.

Table 5 is a summary of the total number of compounds screened from the inception of this program to date.

All compounds tested were obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research and included: (1) compounds structurally related to chemicals of known value as antimalarial agents; (2) compounds structurally unrelated to compounds known to have antimalarial activity; (3) structural analogues of compounds found active in our test system and representing several novel chemical groups.

Our own breeding colony of ICR/HA Swiss mice supplied the large number of animals needed in our tests.

We have continued to use the original test system which was designed specifically to give relatively fast but reliable evaluations from standpoints of antimalarial effect and host toxicity.

This test is based on the responses to candidate compounds by Plasmodium berghei malaria in mice as expressed in comparisons of maximum survival time of treated malaria-infected animals and survival time of untreated malaria-infected controls.

TABLE 1

MONTHLY SCREENING LEVELS

JUNE 1, 1964 - MAY 31, 1965

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE, 1964	763	15,111
JULY, 1964	758	12,810
AUGUST, 1964	593	10,306
SEPTEMBER, 1964	521	8,543
OCTOBER, 1964	558	9,146
NOVEMBER, 1964	612	9,788
DECEMBER, 1964	1,279	20,249
JANUARY, 1965	1,634	25,013
FEBRUARY, 1965	1,399	21,228
MARCH, 1965	1,999	30,831
APRIL, 1965	1,378	23,188
MAY, 1965	1,620	29,502
TOTAL FOR YEAR	13,114	215,715

TABLE 2

MONTHLY SCREENING LEVELS

JUNE 1, 1965 - MAY 31, 1966

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE, 1965	1,545	25,633
JULY, 1965	1,297	19,873
AUGUST, 1965	1,349	20,645
SEPTEMBER, 1965	1,192	18,208
OCTOBER, 1965	1,539	23,515
NOVEMBER, 1965	1,667	25,525
DECEMBER, 1965	1,740	26,650
JANUARY, 1966	2,384	36,503
FEBRUARY, 1966	2,197	33,015
MARCH, 1966	2,613	39,987
APRIL, 1966	2,241	34,395
MAY, 1966	2,967	46,500
TOTAL FOR YEAR	22,731	350,449

TABLE 3

MONTHLY SCREENING LEVELS

JUNE 1, 1966 - MAY 31, 1967

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE, 1966	2,314	36,220
JULY, 1966	2,686	41,175
AUGUST, 1966	2,871	44,825
SEPTEMBER, 1966	2,216	34,420
OCTOBER, 1966	2,644	41,325
NOVEMBER, 1966	2,670	42,285
DECEMBER, 1966	2,712	42,055
JANUARY, 1967	3,048	47,325
FEBRUARY, 1967	3,838	59,970
MARCH, 1967	3,215	49,545
APRIL, 1967	2,886	45,510
MAY, 1967	2,993	46,545
TOTAL FOR YEAR	34,093	531,200

TABLE 4

MONTHLY SCREENING LEVELS

JUNE 1, 1967 - MAY 31, 1958

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE, 1967	3,360	52,485
JULY, 1967	2,629	42,690
AUGUST, 1967	3,222	51,510
SEPTEMBER, 1967	4,174	65,085
OCTOBER, 1967	3,769	58,275
NOVEMBER, 1967	4,255	66,690
DECEMBER, 1967	4,772	73,125
JANUARY, 1968	2,807	43,800
FEBRUARY, 1968	1,679	27,195
MARCH, 1968	3,403	110,923
APRIL, 1968	2,953	113,876
MAY, 1968	3,442	117,318
TOTAL FOR YEAR	40,465	636,525

TABLE 5

MONTHLY SCREENING LEVELS

DECEMBER, 1961 - MAY 31, 1968

DECEMBER, 1961 - NOVEMBER, 1962	250
DECEMBER, 1962 - MAY, 1964	6,665
JUNE, 1964 - MAY, 1965	13,114
JUNE, 1965 - MAY, 1966	22,731
JUNE, 1966 - MAY, 1967	34,093
JUNE, 1967 - MAY 31, 1968	40,465
TOTAL	117,318

Using young ICR/HA Swiss mice and a standard inoculum of Plasmodium berghei, it has been possible to produce a consistently uniform disease that is fatal to 100% of untreated animals within 6 to 8 days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a high degree of parasitemia has become evident.

Test compounds were administered parenterally in a single dose on the third day post-infection by which time a 10-15% parasitemia has developed.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life-span of the treated animals over that of the untreated controls.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds was obtained from our own breeding colony of ICR/HA Swiss mice. Test animals weigh from 15 to 18 grams, weight variations in any given experimental or control group being carefully limited to 2-3 grams. In any given test all animals are of a single sex and approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib.

TEST PROCEDURE. Test animals receive an intraperitoneal injection of 0.5 ml. of 1:100 dilution of heparanized heart's blood with a minimum of 90% parasitized cells, drawn from donor mice infected one week earlier with Plasmodium berghei. The donor strain is maintained by weekly passages in separate groups of mice inoculated with 0.5 ml. of a 1:500 dilution of heparinized heart's blood.

In order to check factors such as changes in the infectivity of our Plasmodium berghei strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at dose levels know to produce definite increases in survival time is included in every experiment as a positive control.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered.

Treatment consists of a single dose given subcutaneously 3 days post-infection. At the time of treatment a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

In each experiment the compound on test is administered in graded doses. Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice.

If an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 60 days are considered as cured.

DRUG ACTIVITY. Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. The minimum effective dose is defined as the minimum dose increasing the life span of treated animals by 100% over the life-span of untreated controls.

An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are subjected to a dose-response curve so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM BERGHEI IN MICE. Of the 40,465 compounds tested from June 1, 1967 through May 31, 1968, over 888 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

COMBINATION STUDIES. 914 combination tests were done. Although some of these gave results indicating slight increases in antimalarial activity, none was sufficiently remarkable to justify further study.

A second procedure, using a different host and parasite and performing reliably either as a confirmatory test or as another primary screen, is a desirable adjunct to any screening program.

We developed a simple but dependable supplementary test that is done with Plasmodium gallinaceum malaria in chicks.

Tables 6, 7, 8* and 9 list, month by month, the number of compounds tested and the number of chicks used in these tests from January, 1965, through May 31, 1968.

Table 10 summarizes the number of compounds tested and the number of chicks used from the inception of this assay system in January, 1965, to date.

Using 9-12 days old chicks and a standard inoculum of Plasmodium gallinaceum, we have been able to produce a consistently uniform disease that is fatal to 100% of untreated controls within 72-96 hours.

In this test, as in our mouse test, the antimalarial activity of candidate compounds is assessed by comparing the maximum survival time of treated malaria-infected chicks and the survival time of untreated malaria-infected controls.

As in the mouse test, a compound has been considered active against malaria if it has produced increases of at least 100% in the survival time of treated chicks over the survival time of untreated controls.

Again as in the mouse test, acceptance of a test compound's antimalarial activity was further predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED).

A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. A minimum effective dose is defined as the minimum dose increasing the life-span of treated animals 100% over the life-span of untreated controls.

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* An outbreak of an avian infectious disease involving entire flocks made it impossible to get the healthy birds that we required, and the chick test was temporarily dropped.

TABLE 6

MONTHLY SCREENING LEVELS

JANUARY 1, 1965 - MAY 31, 1965

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JANUARY, 1965	41	260
FEBRUARY, 1965	94	885
MARCH, 1965	82	1,470
APRIL, 1965	72	1,450
MAY, 1965	86	1,650
TOTAL FOR YEAR	375	5,715

TABLE 7

MONTHLY SCREENING LEVELS

JUNE 1, 1965 - MAY 31, 1966

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE, 1965	94	1,620
JULY, 1965	120	2,020
AUGUST, 1965	166	1,580
SEPTEMBER, 1965	246	1,365
OCTOBER, 1965	464	3,195
NOVEMBER, 1965	179	3,295
DECEMBER, 1965	249	3,465
JANUARY, 1966	197	3,455
FEBRUARY, 1966	163	2,800
MARCH, 1966	202	3,495
APRIL, 1966	264	4,450
MAY, 1966	56	1,195
TOTAL FOR YEAR	2,400	31,935

TABLE 8

MONTHLY SCREENING LEVELS

JUNE 1, 1966 - SEPTEMBER 30, 1966*

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE, 1966	352	5,865
JULY, 1966	334	5,565
AUGUST, 1966	105	2,250
SEPTEMBER, 1966	211	3,540
TOTAL FOR YEAR	1,002	17,220

*An outbreak of an avian infectious disease involving entire flocks made it impossible to get the healthy birds that we required, and the chick test was temporarily dropped.

TABLE 9

MONTHLY SCREENING LEVELS

SEPTEMBER, 1967 - MAY, 1968

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
SEPTEMBER, 1967	90	1,410
OCTOBER, 1967	349	3,330
NOVEMBER, 1967	352	3,150
DECEMBER, 1967	282	2,700
JANUARY, 1968	231	2,400
FEBRUARY, 1968	58	450
MARCH, 1968	367	3,030
APRIL, 1968	698	4,095
MAY, 1968	555	4,290
TOTAL FOR YEAR	2,982	24,855

TABLE 10

MONTHLY SCREENING LEVELS

JANUARY, 1965 - MAY, 1968

JANUARY, 1965 - MAY, 1965		375
JUNE, 1965 - MAY, 1966		2,400
JUNE, 1966 - SEPTEMBER, 1966		1,002
SEPTEMBER, 1967 - MAY 31, 1968		2,982
	TOTAL	6,759

METHOD

TEST ANIMALS. 9-12 days old white Leghorn cockerels of uniform stock were obtained from a single breeder.

The animals were delivered to the laboratory when 1 day old and then maintained under standard conditions, including a non-medicated diet, until they were ready for testing.

TEST PROCEDURE. Chicks on test were given an intrajugular injection of 0.2 ml. of heparinized heart's blood infected with Plasmodium gallinaceum and having a minimum of 80-90% parasitized red blood cells.

The parasitized blood was drawn by cardiac puncture from donor birds that had been infected 72 hours earlier with Plasmodium gallinaceum.

Donor strains are maintained in separate groups of chicks, 14-16 days old, that also receive inoculations of heparinized infected heart's blood.

In every experiment 100% of the untreated controls have died within 72-96 hours post-infection.

In order to check factors such as changes in the infectivity of our Plasmodium gallinaceum strain or in the susceptibility of the host or to detect technical errors, a group of infected birds treated with pyrimethamine at dose levels known to produce definite increases in survival time has been included in every experiment as a positive control.

DRUG ADMINISTRATION. Candidate compounds are dissolved or suspended in peanut oil before they are administered.

In this supplementary test treatment consists of a single dose that is administered either subcutaneously or per os immediately after infection.

Each experiment was done with graded doses of the compound on test, and increases in the dose levels of highly active compounds were generally followed by increases in the survival time of the treated chicks.

If an active drug was toxic for the host, its toxicity became a limiting factor to changes in dosages.

Deaths that occurred within 48 hours after infection and treatment were considered as deaths due to the toxic effects of a test chemical, not as the result of the infection introduced by the Plasmodium gallinaceum parasite.

Chicks with survival periods of 30 days were recorded as cured.

DRUG ACTIVITY. In the chick test, as in the mouse test, an increase of 100% in survival time has been considered as the minimum significantly effective response to the antimalarial activity of a compound.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM GALLINACEUM MALARIA IN CHICKS. Of the 2,982 candidate compounds tested in chicks from September 1, 1967, through May 31, 1968, over 380 demonstrated a degree of antimalarial activity that produced a minimum of 100% increase in the survival time of Plasmodium gallinaceum infected chicks.

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